Impact of silica supplementation on cucumber cultures

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Background

Silicon [Si] is taken up by plants in its soluble form, silicic acid [$Si(OH)_4$] and finally polymerized in the leaves. Even not to be an essential element for plants, its beneficial effects on growth of crops such as rice, barley, wheat, cucumber etc. are reported. Silicic acid treatment decreases the effects caused by abiotic and biotic stresses and it is proposed that silicon also strengthens the mechanical barrier improving the protecting from mycosis. Silicic acid pretreated *Cucumis sativus* plants show improved rapid and extensive defence reaction. It still remains unclear, if such effects also have impact on virus infections.

Our study aims to provide information on changes in gene expression caused by silicic acid treatment of *Cucumis sativus* cultivars. Subsequent studies will allow estimating putative beneficial effects on the prevention of virus infections on cucumber.

Methods

Tissue cultures were generated from the *C. sativus* cultivar Borszczagowski (line B10). Microexplants were prepared for plant regeneration and cultivated on modified Murashige & Skoog-medium supplemented w/ or w/o silicic acid or NaCl (**Fig 1**). In addition, half of the clones were mechanically inoculated with Cucumber Mosaic Virus (CMV) from Germany (PV-0187, DSMZ). Total RNA was isolated from tissue cultures (InviTrap® Spin Plant RNA Mini Kit, Invitek) (**Fig 2**), DNase treated (Rnase-free Dnase I, NEB), mRNA enriched by repeated polyT-oligonucleotide hybridization (Dynabeads® mRNA Purification Kit, Ambion®) (**Fig 3**) and RNA-Seq was performed (Dr. Huettel & Dr. Reinhardt, Max Planck-Genome-Centre Cologne). CLC Genomics Workbench 6.0.2 (CLC Genomics) was used for mapping on the genomic draft of cucumber line B10 (**Fig 4**).

First results

✓Clonal tissue cultures of line B10 were established at the Humboldt-Universität zu Berlin and subsequently cultivated on regeneration medium.

✓Infection experiments using Cucumber Mosaic Virus were successfully applied in greenhouse and micro-propagation experiments.

✓Silicic acid treated cultures and controls provided enriched mRNA samples that were used for subsequent RNA-Seq (**Fig 5**).

✓Initial analysis allowed the identification of above 18,000 cucumber genes. Analysis of observed shifts in expression and its confirmation by quantitative (q) reverse transcription (RT)-PCR is in progress.

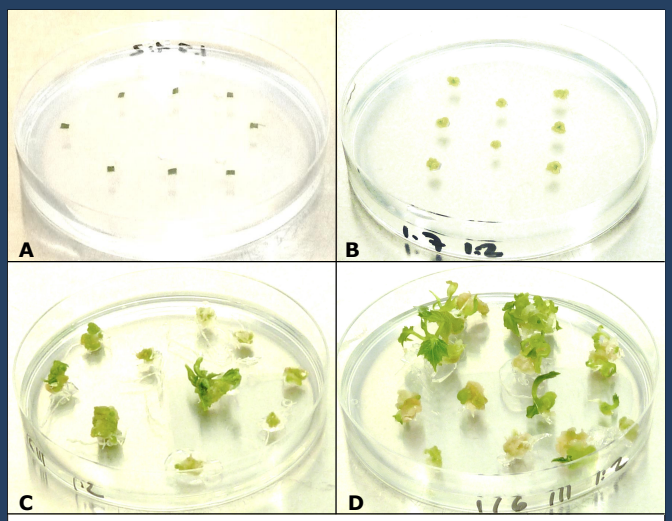


Figure 1. Different stages of B10 regeneration on Murashige & Skoog-medium. Images show calli and regenerants cultivated on control medium: **A** Prepared leaf microexplants from the first leaf on induction medium prior to cultivation in the dark. **B** Early regeneration stage after exposition to light (2 weeks of culture). **C** Calli and young shoots on regeneration medium (6 weeks of culture). **D** Calli and shoots with leaves (8 weeks of culture), prior to transfer to rooting medium.

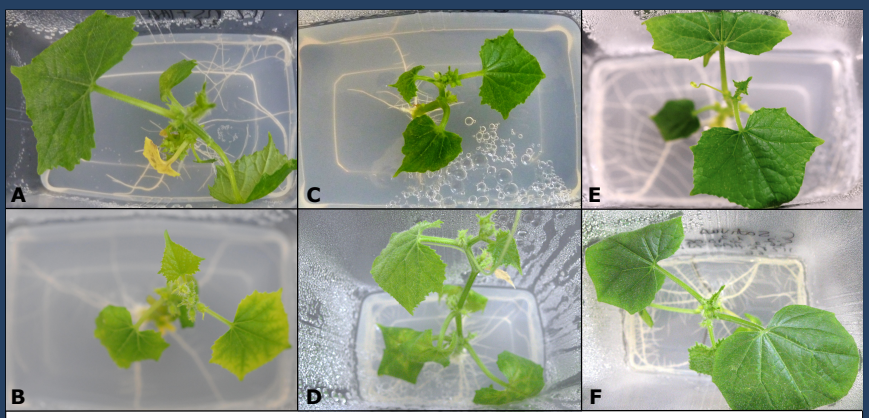


Figure 2. Differentiated B10 clones. All regenerants were cultivated on modified Murashige & Skoog-medium containing indole acetic acid for rooting and different treatments, respectively. Images show regenerants (12 weeks of cultivation) prior to sampling for RNA isolation: **A** Regenerant on control medium. **B** Regenerant on control medium and infected with Cucumber Mosaic Virus (CMV). **C** Regenerant treated with 2 mM NaCl. **D** Regenerant treated with 2 mM NaCl and infected with CMV. **E** Regenerant treated with 2 mM silicic acid. **F** Regenerant treated with 2 mM silicic acid and infected with CMV.

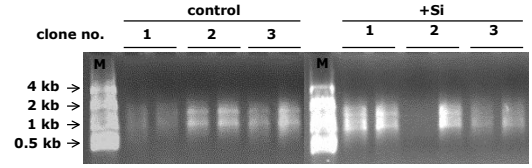
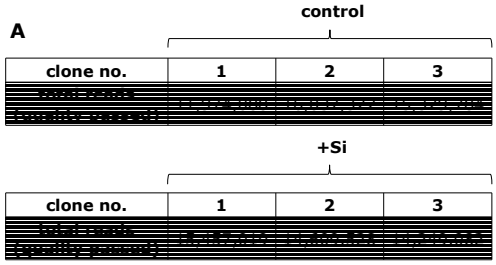


Figure 3. Enriched mRNA B10 clones cultivated *in vitro* in absence (control) or presence (+Si) of silicon. Per clone, 4 total RNA isolations were conducted from leaves and stems. After quality and concentration check, the samples were pooled and combined to 2 samples per individual clone. DNase digestion was performed followed by mRNA enrichment.



=> mapped to reference draft genome *Cucumis sativus* B10 consisting of:

- 323,986,325 bp sequence length
- 13,113 scaffolds
- 20,327 genes
- 19,782 transcripts

B **=> in average: each predicted gene is assigned to 1 transcript**

	control (A)	+Si (B)
mapped on genes	11,541,189	13,029,551
[illegible]	[illegible]	[illegible]
expressed genes	18,069	18,007

- **1180 differential expressed genes**
- **differential expression analysis is in progress**
- **recalculation is in progress**

Figure 5. First experiment on silicic acid supplementation of cucumber line B10 clones (differential expression). Single reads were obtained with an average read length of 94 b. All analyses were conducted using CLC Genomics Workbench: **A** Overview of all reads. **B** Overview of mapped sets.

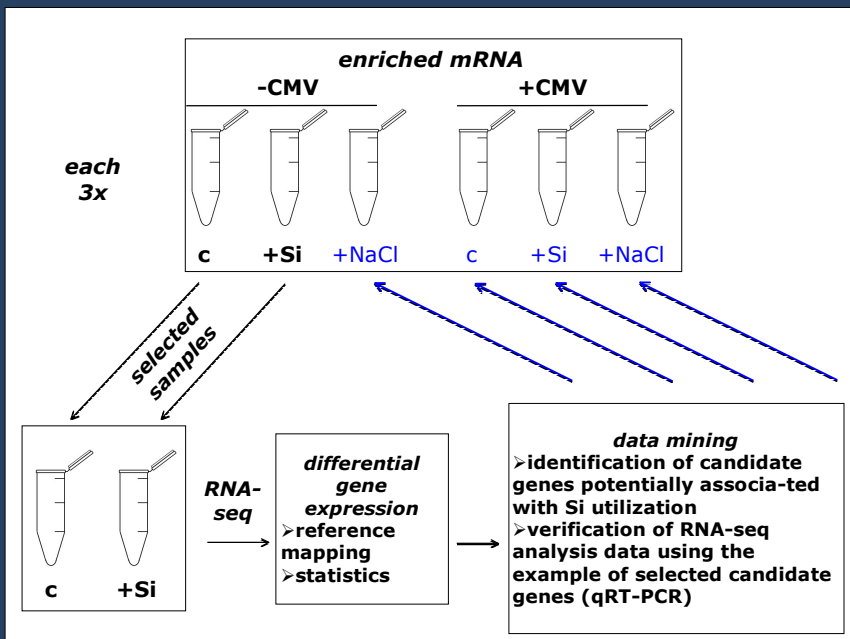


Figure 4. Experimental set-up. *Cucumis sativus* clones were cultivated. Per treatment, 3 clones were obtained (18 clones in total). Control plants (c) were cultivated on non modified medium, whereas 2 mM silicic acid (+Si) and 2 mM NaCl respectively were added to Murashige & Skoog-medium. Half of the clones were mechanically inoculated with Cucumber Mosaic Virus (CMV). 6 days after inoculation, samples from all clones were taken for RNA isolation followed by mRNA enrichment. mRNA samples were taken from the non infected control- and Si-group (6 samples in total) for RNA-seq performed with the Illumina platform (black). Reference mapping to B10 draft genome and differential gene expression was calculated using CLC Genomics Workbench. Genes associated with Si utilization shall be analyzed in the other 4 treatment groups using mRNA for quantitative (q) reverse transcription (RT)-PCR (blue).

Acknowledgments

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